

Appl. No. : 09/853,080
Filed : May 9, 2001

Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg ,
possibly deleted from one or more amino acids of the epitope Asp-Glu-Asp-Glu (SEQ ID
NO: 35),

the epitope threonine 1739 to tyrosine 1748 inclusive, defined by the following
sequence:

SEQ ID NO:3:

Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr ,

the epitope asparagine 1777 to phenylalanine 1785 inclusive, defined by the following
sequence:

SEQ ID NO:4:

Asn Gln Ala Ser Arg Pro Tyr Ser Phe ,

possibly deleted from one or two amino acids of the terminal dipeptide Ser-Phe or the
tetrapeptide Pro-Tyr-Ser-Phe (SEQ ID NO: 36),

the epitope glutamic acid 1794 to tyrosine 1815 inclusive, defined by the following
sequence:

SEQ ID NO:5:

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr
Tyr ,

possibly deleted from one or more amino acids from the first tripeptide Glu-Asp-Gln
(SEQ ID NO: 37) or the first nonapeptide Glu-Asp-Gln-Arg-Gln-Gly-Ala-Glu-Pro (SEQ ID NO:
38),

the epitope methionine 1823 to aspartic acid 1831, defined by the following sequence:

SEQ ID NO:6:

Met Ala Pro Thr Lys Asp Glu Phe Asp ,

the epitope glutamic acid 1885 to phenylalanine 1891 inclusive, defined by the following
sequence:

SEQ ID NO:7:

Glu Thr Lys Ser Trp Tyr Phe ,

the epitope glutamic acid 1885 to alanine 1901 inclusive, defined by the following
sequence:

SEQ ID NO:8:

Glu Thr Lys Ser Trp Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala ,

possibly deleted from one or more amino acids from the heptapeptide Gly-Thr-Lys-Ser-
Trp-Phe-Thr (SEQ ID NO: 39) or from the tripeptide Cys-Arg-Ala (SEQ ID NO: 40),

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the epitope aspartic acid 1909 to arginine 1917 inclusive, defined by the following sequence:

SEQ ID NO:9:

Asp Pro Thr Phe Lys Glu Asn Tyr Arg ,

and the epitope comprised between serine 2018 and histidine 2031 inclusive, defined by the following sequence:

SEQ ID NO:10:

Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His .

Paragraph 8: An antigenic fragment of the polypeptide sequence A1 according to paragraph 5, which is alanine 108 to methionine 355 inclusive, preferably alanine 108 to alanine 227 inclusive.

Paragraph 9: A sequence epitope of the fragment according to paragraph 8, which is selected from the group consisting of:

the epitope alanine 108 to valine 128 inclusive, defined by the following sequence:

SEQ ID NO:11:

Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val ,
possibly deleted from the terminal amino acids alanine and/or valine,

the epitope glutamic acid 181 to leucine 192 inclusive, defined by the following sequence:

SEQ ID NO:12:

possibly deleted from one or two amino acids of the terminal dipeptide Thr-Leu,
the epitope aspartic acid 203 to alanine 227 inclusive, defined by the following

sequence:

SEQ ID NO:13:

Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala
Ser Ala Arg Ala,

possibly deleted from one or more amino acids of the nonapeptide Asp-Arg-Asp-Ala-Ala-Ser-Ala-Arg-Ala (SEQ ID NO: 41),

and the epitope aspartic acid 327 to methionine 355 inclusive, defined by the following sequence:

SEQ ID NO:14:

Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr
Asp Asp Asp Leu Thr Asp Ser Glu Met ,

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possibly deleted from one or more amino acids of the dipeptide Asp-Ser or the octapeptide Asp-Asp-Leu-Thr-Asp-Ser-Glu-Met (SEQ ID NO: 42),

Paragraph 10: An antigenic fragment of the antigenic polypeptide sequence A2 according to paragraph 5, which is aspartic acid 403 to aspartic acid 725 inclusive, preferably histidine 693 to aspartic acid 725 inclusive.

Paragraph 11: A sequence epitope of the fragment according to paragraph 10, which is selected from the group consisting of:

the epitope aspartic acid 403 to lysine 425 inclusive, defined by the following sequence:

SEQ ID NO:15:

Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys
Tyr Lys Lys ,

possibly deleted from one or more amino acids of the tetrapeptide Asp-Asp-Arg-Ser
SEQ ID NO: 43),

the epitope valine 517 to arginine 527 inclusive, defined by the following sequence:

SEQ ID NO:16:

Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg ,

possibly deleted from one or the two amino acids of the dipeptide Pro-Arg,

the epitope tyrosine 555 to glutamine 565 inclusive defined by the following sequence:

SEQ ID NO:17:

Tyr Lys Glu Ser Val Asp Gly Arg Gly Asn Gln ,

the epitope histidine 693 to glycine 701 inclusive, defined by the following sequence:

SEQ ID NO:18

His Asn Ser Asp Phe Arg Asn Arg Gly ,

the epitope serine 710 to aspartic acid 725 inclusive, defined by the following sequence:

SEQ ID NO:19

Ser Cys Asp Lys Asn Thr Gly Asp Tyr Try Gly Asp Ser Tyr Glu Asp ,

the epitope leucine 730 to serine 741 inclusive, defined by the following sequence:

SEQ ID NO:20:

Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser ,

possibly deleted from the terminal amino acid serine and/or the first amino acid leucine,

the epitope serine 817 to serine 830 inclusive, defined by the following sequence:

SEQ ID NO:21:

Ser Asp Asp Pro Ser Gly Ala Ile Asp Ser Asn Asn Ser .

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Paragraph 12: An antigenic fragment of the antigenic polypeptide sequence C according to paragraph 5, which is lysine 2085 to isoleucine 2251 inclusive, or leucine 2273 to tyrosine 2332 inclusive, preferably lysine 2085 to glycine 2121 inclusive or serine 2182 to leucine 2251 inclusive.

Paragraph 13: A sequence epitope of the fragment according to paragraph 12, which is selected from the group consisting of:

the epitope isoleucine 2081 to serine 2095 inclusive, defined by the following sequence
SEQ ID NO:22:

Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser ,
possibly deleted from one or more amino acids of the tetrapeptide Ile-His-Gly-Ile (SEQ ID NO: 44),

the epitope tyrosine 2105 to glycine 2121 inclusive, defined by the following sequence:
SEQ ID NO:23:

Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly ,
possibly deleted from one or more amino acids of the tripeptide Tyr-Ser-Leu (SEQ ID NO: 45),

the epitope asparagine acid 2128 to asparagine acid 2138 inclusive, defined by the following sequence:

SEQ ID NO:24:

Asn Val Asp Ser Ser Gly Ile Lys His Asn ,

the epitope histidine 2152 to arginine 2163 inclusive, defined by the following sequence:
SEQ ID NO:25:

His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg ,

the epitope serine 2181 to asparagine acid 2198 inclusive, defined by the following sequence:

SEQ ID NO:26:

Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn ,
possibly deleted from one or more amino acids of the first dipeptide Ser-Tyr or one or more amino acids from the terminal tripeptide Phe-Thr-Asn (SEQ ID NO: 46),

the epitope serine 2204 to glutamine 2222 inclusive, defined by the following sequence:
SEQ ID NO:27:

Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln,

the epitope glutamine 2235 to leucine 2251 inclusive, defined by the following sequence:
SEQ ID NO:28:

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Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu ,
possibly deleted from one or two amino acids of the terminal dipeptide Ser-Leu or one or
more amino acids of the tetrapeptide Val-Lys-Ser-Leu (SEQ ID NO: 47),

the epitope glycine 2242 to leucine 2251 inclusive, defined by the following sequence:

SEQ ID NO:29:

Gly Val Thr Thr Gln Gly Val Lys Ser Leu ,

possibly deleted from one or two amino acids of the terminal dipeptide Ser-Leu,

the epitope isoleucine 2262 to glutamine 2270 inclusive, defined by the following sequence:

SEQ ID NO:30:

Ile Ser Ser Ser Gln Asp Gly His Gln ,

the epitope leucine 2273 to serine 2289 inclusive, defined by the following sequence:

SEQ ID NO:31:

Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser ,

the epitope proline 2292 to tyrosine 2305 inclusive, defined by the following sequence:

SEQ ID NO:32:

Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr ,

possibly deleted from one or more amino acids of the terminal tripeptide Thr-Arg-Tyr
(SEQ ID NO: 48),

**Please replace the paragraphs beginning on page 17 at line 25 through page 23,
line 28, with the following rewritten paragraphs:**

SEQ ID NO.:1:

Arg Asp Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr ,

and possibly deleted from one or more amino acids of the tetrapeptide Arg-Asp-Ile-Thr
(SEQ ID NO: 34) (P7), or one or two of the last amino acids of the dipeptide Asp-Tyr

- the epitope aspartic acid 1681 to arginine 1696 (P8) inclusive, defined by the following
sequence:

SEQ ID NO.:2:

Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg

possibly deleted from one or more amino acids of the epitope Asp-Glu-Asp-Glu (SEQ ID
NO: 35),

- the epitope threonine 1739 to tyrosine 1748 inclusive, defined by the following sequence:

SEQ ID NO.:3:

Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr

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- the epitope asparagine 1777 to phenylalanine 1785 inclusive, defined by the following sequence:
SEQ ID NO.:4:
Asn Gln Ala Ser Arg Pro Tyr Ser Phe
possibly deleted from one or more amino acids of the terminal dipeptide Ser-Phe or tetrapeptide Pro-Tyr-Ser-Phe (SEQ ID NO: 36),
- the epitope glutamic acid 1794 to tyrosine 1815 inclusive, defined by the following sequence:
SEQ ID NO.:5:
Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro
Asn Glu Thr Lys Thr Tyr ,
possibly deleted from one or more amino acids of the first tripeptide Glu-Asp-Gln (SEQ ID NO: 37 (P9)) or the first nonapeptide Glu-Asp-Gln-Arg-Gln-Gly-Ala-Glu-Pro (SEQ ID NO: 38),
- B2 - the epitope methionine 1823 to aspartic acid 1831 inclusive, defined by the following sequence:
SEQ ID NO.:6:
Met Ala Pro Thr Lys Asp Glu Phe Asp
- the epitope glutamic acid 1885 to phenylalanine 1891 inclusive, defined by the following sequence:
SEQ ID NO.:7:
Glu Thr Lys Ser Trp Tyr Phe
- the epitope glutamic acid 1885 to alanine 1901 inclusive, defined by the following sequence:
SEQ ID NO.:8:
Glu Thr Lys Ser Trp Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala
possibly deleted from one or more amino acids from the heptapeptide Glu-Thr-Lys-Ser-Trp-Phe-Thr (SEQ ID NO: 39) or from the tripeptide Cys-Arg-Ala (SEQ ID NO: 40).
- the epitope aspartic acid 1909 to arginine 1917 inclusive, defined by the following sequence:
SEQ ID NO.:9:
Asp Pro Thr Phe Lys Glu Asn Tyr Arg
- the epitope comprised between serine 2018 and histidine 2031 inclusive, defined by the following sequence:
SEQ ID NO.:10:

Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His

Advantageously, the said sequences, specific fragments and epitopes exhibit an antigenic characteristic which is illustrated by Table 1.

Another preferred embodiment of the invention relates to antigenic polypeptide sequence A1 of factor VIII, fragments and/or epitopes of this sequence.

Preferably, the fragments of the said sequence are alanine 108 to methionine 355 inclusive, preferably alanine 108 to alanine 227 inclusive.

The invention also relates to the sequence epitopes of these fragments, in particular:

- the epitope alanine 108 to valine 128 inclusive, defined by the following sequence:

SEQ ID NO.:11:

Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys

Glu Asp Asp Lys Val

possibly deleted from the terminal amino acids alanine and valine (P1)

- the epitope glutamic acid 181 to leucine 192 inclusive, defined by the following sequence:

SEQ ID NO.:12:

Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu

possibly deleted from one or two amino acids of the terminal dipeptide Thr-Leu

- the epitope aspartic acid 203 to alanine 227 inclusive, defined by the following sequence:

SEQ ID NO.:13:

Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala

Ser Ala Arg Ala

possibly deleted from one or more amino acids of the nonapeptide Asp-Arg-Asp-Ala-Ala-Ser-Ala-Arg-Ala (SEQ ID NO: 41)

- the epitope aspartic acid 327 to methionine 355 inclusive, defined by the following sequence:

SEQ ID NO.:14:

Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp

Asp Asp Leu Thr Asp Ser Glu Met

possibly deleted from one or more amino acids from the terminal dipeptide Asp-Ser or the octapeptide Asp-Asp-Leu-Thr-Asp-Ser-Glu-Met (SEQ ID NO: 42 (P2)).

Another preferred embodiment of the invention relates to the antigenic polypeptide sequence A2 of factor VIII, fragments and/or epitopes of this sequence.

Preferably, the fragments of the said sequence are aspartic acid 403 to serine 840 inclusive, preferably histidine 693 to aspartic acid 725 inclusive.

The invention also relates to the sequence epitopes of these fragments, in particular:

- the epitope aspartic acid 403 to lysine 425 inclusive, defined by the following sequence:
SEQ ID NO.:15:
Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg
Ile Gly Arg Lys Tyr Lys Lys
possibly deleted from one or more amino acids of the tetrapeptide Asp-Asp-Arg-Ser (SEQ
ID NO: 43 (P3)),
- the epitope valine 517 to arginine 527 inclusive, defined by the following sequence:
SEQ ID NO.:16:
Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg
possibly deleted from one or the two amino acids of the dipeptide Pro-Arg,
- the epitope tyrosine 555 to glutamine 565 inclusive, defined by the following sequence:
SEQ ID NO.:17:
Tyr Lys Glu Ser Val Asp Gly Arg Gly Asn Gln
- the epitope histidine 693 to glycine 701 inclusive, defined by the following sequence:
SEQ ID NO.:18:
His Asn Ser Asp Phe Arg Asn Arg Gly
- the epitope serine 710 to aspartic acid 725 inclusive, defined by the following sequence
(P4):
SEQ ID NO.:19:
Ser Cys Asp Lys Asn Thr Gly Asp Tyr Try Gly Asp Ser Tyr Glu Asp
the epitope leucine 730 to serine 741 inclusive, defined by the following sequence (P4):
SEQ ID NO.:20:
Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser
possibly deleted from the terminal amino acid serine (P4) and/or the first amino acid
leucine
- the epitope serine 817 to serine 830 inclusive, defined by the following sequence (P5):
SEQ ID NO.:21:
Ser Asp Asp Pro Ser Gly Ala Ile Asp Ser Asn Asn Ser

A final preferred embodiment of the invention relates to the antigenic polypeptide sequence C of factor VIII, and fragments and/or epitopes of this sequence. Preferably, the fragments of the said sequence are histidine 2082 to lysine 2251 inclusive or leucine 2273 to tyrosine 2332 inclusive, preferably lysine 2085 to glycine 2121 inclusive and serine 2181 to leucine 2251 inclusive.

The invention also relates to the sequence epitopes of these fragments, in particular:

- the epitope isoleucine 2081 to serine 2095 inclusive, defined by the following sequence:
SEQ ID NO.:22:
Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser
possibly deleted from one or more amino acids from the tetrapeptide Ile-His-Gly-Ile (SEQ ID NO: 44)
- the epitope tyrosine 2105 to glycine 2121 inclusive, defined by the following sequence:
SEQ ID NO.:23:
Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr
Gly
possibly deleted from one or more amino acids of the tripeptide Tyr-Ser-Leu (SEQ ID NO: 45 (P10))
- the epitope asparagine 2128 to asparagine 2138 inclusive, defined by the following sequence:
SEQ ID NO.:24:
Asn Val Asp Ser Ser Gly Ile Lys His Asn
the epitope histidine 2152 to arginine 2163 inclusive, defined by the following sequence:
SEQ ID NO.:25:
His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg
the epitope serine 2181 to asparagine 2198 inclusive, defined by the following sequence:
SEQ ID NO.:26:
Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn
possibly deleted from one or more amino acids from the terminal tripeptide Phe-Thr-Asn (SEQ ID NO: 46 (P11))
- the epitope serine 2204 to glutamine 2222 inclusive, defined by the following sequence (P12):
SEQ ID NO.:27:
Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp
Arg Pro Gln
- the epitope glutamine 2235 to leucine 2251 inclusive, defined by the following sequence (P13):
SEQ ID NO.:28:
Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu